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Viral mechanisms for docking and delivering at nuclear pore complexes

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Abstract

Some viruses possess the remarkable ability to transport their genomes across nuclear pore complexes (NPCs) for replication inside the host cell's intact nuclear compartment. Viral mechanisms for crossing the restrictive NPC passageway are highly complex and astonishingly diverse, requiring in each case stepwise interaction between incoming virus particles and components of the nuclear transport machinery. Exactly how a large viral genome loaded with accessory proteins is able to pass through the relatively narrow central channel of the NPC without causing catastrophic structural damage is not yet fully understood. It appears likely, however, that the overall structure of the NPC changes in response to the cargo. Translocation may result in nucleic acids being misdelivered to the cytoplasm. Here we consider in detail the diverse strategies that viruses have evolved to target and subvert NPCs during infection. For decades, this process has both captivated and confounded researchers in the fields of virology, cell biology, and structural biology.

1. Introduction

Virus particles from the inside out are composed of a core of nucleic acids and accessory factors, a bridge of cement-like capsid proteins, and a multifunctional coat that is sometimes covered in lipids and sugars [1, 2]. This hierarchy of metastable assembly networks intricately connects to form the virion. During cell entry, virions break down in a controlled, stepwise, gain-of-function process termed uncoating that aims to safely deliver the viral genome to the site of replication, which for this review is the nucleus [3-6] (see Fig. 1 for generic entry).

Uncoating programs, like viral nuclear import, are complex and uniquely defined by the built-in mechanisms of the invading virion and the make-up and components of the target host cell. Regulated disassembly begins once a virus has located and firmly attached to the plasma membrane of a compatible cell by binding surface structures known as attachment factors and receptors [7]. Interactions with attachment factors are relatively nonspecific and serve to concentrate capsids at the surface of the cell, whereas receptor interactions are specific, induce conformational changes in the virion, and promote formation of glycolipoprotein microdomains that signal for internalization [8]. Viral capsids harbor multiple binding sites and will often engage more than one receptor in parallel or in series, which results in nearly irreversible binding. At the same time this helps to recruit the cellular uptake machinery.

To move beyond the cell periphery, virus particles, often with receptors in tow, usurp endocytic pathways, which they exit in a timely manner to avoid lysosomal degradation [9]. Virion-carrying vesicles undergo maturation as they travel towards the perinuclear region. During this vesicular residence, confined capsids are exposed to chemical cues (e.g. low pH), facilitators (e.g. endosomal maturation factors), and topographical features that permit expedient escape to the cytoplasm [7]. Penetration of vesicle membranes, for enveloped viruses, involves membrane fusion, and for nonenveloped viruses, it involves pore formation or membrane lysis [10, 11]. Now the partially disassembled virus particles are in yet another environment, the cytosol, and there access the microtubule transport system, or move by other means, such as actin polymerization, to reach the nuclear envelope (NE) and eventually the NPC. It is at this time that intracellular uncoating factors often execute the final steps of disassembly so that genomes can be released for transport into the nucleus (see Table 1 summary).

2. The NPC as a selective gate to the nucleus

NPCs function as selective, bidirectional portals that span the two membranes of the lamin-fortified NE in a way that allows control over transport into and out of the nucleus [12-14]. NPCs (~ 2000-3000 per nucleus) organize randomly at the surface of the nucleus as octameric rings of protein via an inside-out extrusion mechanism that begins with inner membrane deformation and ends after fusion with the outer layer of the NE [15]. Assembly

involves the joining together of biochemically distinct and notably stable subcomplexes, the largest and best characterized being the Y complex, which serves as the essential scaffolding module of the NPC. The Y complex is anchored to the NE by a few different transmembrane Nups, and with the help of further scaffold and adaptor Nups, establishes the disordered phenylalanine-glycine (FG) permeability barrier located at the central passageway [16].

Organized in this fashion, scaffold Nups have been shown to remain stably associated, with residence times of hours or days, whereas FG and other Nups have dwell times in the range of minutes [17, 18]. All in all, the rigid elements of the NPC architecture are held together by flexible linkers to form a complex that is ~ 110 nm wide x ~ 70 nm tall, and that has an unstructured central channel measuring ~ 40 nm in diameter, also referred to as the pore [19, 20]. Such space allows ions, metabolites, and small macromolecules no bigger than about 5 nm or roughly 40 kDa in size to diffuse freely, but requires that larger cargoes use receptor assistance for efficient nuclear import [21].

Three Nups have been mapped to the cytoplasmic edge of the pore. Nup214 and Nup88 reside near the cytoplasmic opening of the pore. Purified Nup358 forms flexible ~ 36 nm long filaments and in the NPC is the main constituent of extensions that emanate into the perinuclear cytoplasmic region. On the nuclear side, eight filaments composed of Tpr, Nup50, and Nup153 join together to make a basket structure.

NPCs are the most efficient traffic controllers found in nature, granting fast passage at a rate of ~ 1000 translocation events per second for small molecules and transport receptor (TR) bound cargoes, while at the same time rejecting nonspecific, wandering macromolecules [22]. Small molecule (< 40 kDa) movement into the nucleus is relatively straightforward, apparently requiring no input of chemical energy or assistance from soluble factors. Inappropriate cytoplasmic proteins that leak into the nucleus are efficiently recognized and expelled by CRM1 in an energy-dependent manner [23].

Larger-sized cargo needs an appropriate peptide NLS, and essential transporters (e.g., importins, exportins, transportins) and regulators (e.g., the small Ran GTPase) to guide NPC translocation [24]. Transporters recognize NLSs and form TR-cargo complexes, which upon associating have a high capacity to interact with FG Nups located at the periphery and lining the inside of the NPC. Regulators work in parallel at the NPC to modulate permeability, establish directionality, and release translocated cargo. The Ran GTPase tunes interactions that build or break TR-cargo complexes in a compartment specific manner. Recent experiments indicate that Ran itself can influence the permeability barrier of the NPC by dissolving a Nup153-impβ mesh located along the transport axis near the nuclear face [25].

The entire process of TF-cargo docking, passage, and release typically occurs on the order of a few milliseconds [26-28]. Conceptualizing and experimentally testing NPC transport models has proved exceedingly difficult, in large part because of the unstructured-barrier Nups tethered throughout the pore. Not surprisingly, several models have been proposed to explain translocation speed and selectivity. In brief, it is believed that FG Nups achieve fast and selective transport either by organizing cohesively to form a sieve-like hydrogel, by functioning as repulsive bristles that entropically exclude, by collapsing in the

presence of TR-cargo complexes, or perhaps by a hybrid thereof [29-37]. From here on, we shift the focus to viruses, which have evolved mechanisms for nuclear import of their genomes. We survey the unique strategies that they use to import their large-sized genomes across NPCs.

3. Viral NPC transport mechanisms: shape fitting and forced break-in

Viruses that make use of the host cell`s nuclear compartment as a site for replication navigate the central channel of the NPC, or in the case of most retroviruses and papillomaviruses, rely on cell division to remove the NE barrier for nuclear transcription and replication [38-43]. The central channel of the NPC is a dynamic, selective, size-restricted, and crowded environment that at any given moment is loaded with fast moving cargo. That a virus can deliver a relatively large hydrophilic genome across a tight hydrophobic space and against a steep concentration gradient of host chromatin is an impressive feat, requiring a high level of coordination.

Preparation for nuclear import begins the moment a virus binds at the cell surface and is internalized, with the entry route and viral coat ultimately setting the stage for how the incoming genome gets translocated. Virologists have put great effort into deciphering viral nuclear transport mechanisms, which differ in detail, but can be separated into two main categories: shape fitting and forced break-in. Shape fitting, much like it sounds, fits the shape of virus particles (HBV, AcMNPV, and AAV2), or subviral structures (IAV and SV40) to the shape of the NPC central passageway for nuclear entry. Forced break-in, on the other hand, occurs at the cytoplasmic opening of the pore, and involves tension and tugging to release encapsidated genomes (Ad5, HSV1, and HIV1) for import into the nucleus.

Regardless of the pathway for intrusion, bulky viral genomes push the limits of transport capacity and increase the chances that something goes wrong with respect to NPC trafficking (for a first review on viral misdelivery at the NPC see [44]).

Below we survey viral NPC transport mechanisms for eight viruses, covering in each case the current understanding of how they dock and deliver genomes into the nucleus for replication (Fig. 2). Additionally, where data is available, we will comment on the topic of genome misdelivery at the NPC, which in recent years has emerged as a potential major bottleneck for viruses during the entry phase of infection.

3.1 Adenovirus Types 2 and 5

The virion organization of Ad2 (human adenovirus species C type 2) and Ad5, which are very similar in terms of genetic makeup and infection biology, but have a distinct serology, is complex. The virion consists of three major (hexon, penton, and fiber) and four minor (IIIa, VI, VIII and IX) proteins that fit together to form a striking icosahedral shape [45, 46]. At the core of the nonenveloped capsid shell is a linear ~ 36 kb double-stranded DNA genome accompanied by five accessory proteins (V, VII, X, IVa2, and terminal protein) and viral protease. The core condenses into an unstructured fluid-like state based on cryo-ET maps of single, intact, nonicosahedrally averaged adenovirus particles analyzed by MD simulations

[47]. Virions are initially stiff with an internal pressure estimated at approximately 30 atm and start loosening at the five-fold vertex regions upon association with integrin αvβ5 [48, 49].

During entry, fiber, penton base, and minor proteins IIIa, VI, and VIII are released from the capsid [50]. To access the cytosol, incoming virions tune lipid composition at the plasma membrane and in endosomes [51]. Tuning is initiated by Ad2/5 engagement of receptors at the cell surface, which is accompanied by release of several copies of protein VI. Release of membrane lytic protein VI induces small lesions in the plasma membrane that trigger a host lysosomal exocytosis repair response resulting in release of acid sphingomyelinase and degradation of sphingomyelin to ceramide lipids. A ceramide-enriched membrane enhances viral endocytosis and boosts the ability of protein VI to rupture endosomes as shown by experiments with synthetic and cell-derived liposomes, and virion uptake assays. Single cell and single virion uptake assays showed that Ad2/5 penetrates into the cytosol independent of low endosomal pH [52]. Virions in the cytosol are then able to recruit dynein for transport to the nucleus [50, 53-56].

Once at the NE barrier, partially disassembled Ad2/5 capsids bind to NPCs via an interaction between the viral capsid protein hexon and an N-terminal region of cytoplasmically oriented Nup214 [57-59]. This allows the mega-dalton virus particle to latch on to the pore complex. Yet, the transport process is far from over as the ~ 92 nm capsid will not fit inside the 40 nm wide central channel, and hence so-called forced break-in is required. Here, outer surface minor capsid protein IX recruits the light chain of a kinesin-1 motor to Nup214-anchored virus, whereafter Nup358, which is adjacent to Nup214, initiates a forceful tug by activating the Kif5C heavy chain for motor movement on microtubules [60]. The resulting pull is strong enough to disassemble the virion on the spot and release the inner core polymer of DNA with associated protein VII and terminal protein for transport through NPCs. The loosely genome-associated protein V is released before DNA import into the nucleus [61][62]. Incoming Ad5-DNA labeled with ethynyl-modified cytosine (EdC) and adenosine (EdA) was the first incoming viral genome measured at single molecule resolution [63]. These studies showed that the incoming Ad5 capsids discharge their DNA contents upon reaching the NPC. Viral DNA translocation is supported by the NLS-containing domains of protein VII, which can strongly bind to nuclear transport receptors including importin-a, importin-b, importin-7, and transportin [64]. Notably, kinesin-1 mediated disassembly dislodges Nup214, Nup358, and Nup62 from the NPC, and in many cases viral DNA accumulates capsid-free in the cytosol [60, 65]. Misdelivery in this context may result from improper capsid docking or uncoating at the NPC, because of difficulties related to translocating the large and unwieldy fluid-like viral genome, or perhaps if the structural integrity of the transport passageway becomes compromised [44].

3.2 Influenza A Virus

Influenza A virus (IAV) packages all of the viral components that are essential and sufficient for transcription and replication of its negative-sense, single-stranded RNA genome into eight segments, referred to as ribonucleoprotein (RNP) particles. Each RNP contains one copy of the heterotrimeric RNA-dependent RNA polymerase and the viral RNA wrapped

around oligomerized nucleoprotein (NP). Cryo-EM reconstructions have clarified that each segment folds into a double-stranded helical hairpin structure with helical turns containing around 120 to 150 RNA nucleotides. Major and minor grooves are supported by NPs throughout, and a single copy of the viral polymerase resides at the open end of the RNA hairpin [66, 67].

After being internalized, the eight RNPs, which are initially bundled together inside of incoming virion, get released from endocytic vesicles by the coordinated activities of the envelope glycoprotein HA and the M2 proton channel [68]. M2 renders the RNP core uncoating-competent by acidifying the M1 interior [69, 70]. In addition, M2 supports the conductance of potassium ions, and further weakens the virion interior so that RNPs are released after fusion with the limiting endosomal membrane [71-73]. The virion capsid protein M1 contains free C-terminal ubiquitin that is recognized by HDAC6 [74]. HDAC6 serves as an adaptor for dynein-mediated transport of misfolded proteins towards the aggresomal degradation machinery and has also been shown to bind myosin II [75, 76]. In the context of IAV entry, it is thought that dynein pulls against a holding force by actomyosin and thereby leads to the disruption and dispersal of the M1 coat [74]. This is akin to a previously described mechanism for the disruption of Ad5 capsids by kinesin-1, which works against the holding force of the NPC [58, 60]. It is also akin to the stripping of the fibers from the incoming Ad5 particles at the plasma membrane by the pulling force of acto-myosin latching to the fiber receptor CAR (coxsackievirus adenovirus receptor) against the holding force of integrins binding to the capsid penton base [77]. Newly penetrated IAV RNPs thereby dissociate from M1 either as smaller subsets, or as individual rod-shaped segments, which are of the appropriate dimensions (a single rod is 10-15 nm in width by 30-120 nm in length) for passage through the NPC [78].

Cytosolic RNPs appear to rely mainly on diffusive movement and multiple NLSs to dock and traffic through NPCs. Notably, each of the subunits of the polymerase bears NLS motifs, but it is the surface exposed 13 amino acid nonclassical NLS of NP, which is periodically displayed in high abundance, and binds different importin-a isoforms that is likely the dominant nuclear trafficking signal [79-87].

Once importin-a adaptor proteins are in place, importin-b is recruited for RNP escort through the NPC central channel [88, 89]. Upon entering the nucleus, Ran-GTP binds and triggers release of the RNPs from TRs [90]. Interestingly, export studies have shown that IAV degrades Nup153, downregulates Nup98, and dislodges Nup62 for genome transport, suggesting that despite having a favorable shape, RNP transport places a considerable amount of stress on the NPC [91-93]. How this stress impacts import efficiency is not yet know, but live cell single-particle tracking experiments have revealed heterogeneous interactions between RNPs and NPCs with dwell times ranging from 1 to 100 seconds [94]. Such rate constants imply that at the least, IAV stalls at the NPC given that cellular cargo transport normally falls within the range of a few milliseconds [26].

3.3 Herpes Simplex Virus Type 1

Herpes simplex type 1 (HSV1) is an enveloped virus with a 125 nm icosahedral capsid, which protects a nucleoprotein core containing the 152 kbp linear double-stranded DNA viral genome. The T=16 capsid, which adopts the canonical HK97 fold, is built of the major structural protein VP5, which assembles into 150 hexons to form the shell and 11 pentons to pack at 11 vertices, while the 12th vertex contains the dodecameric pUL6 portal complex [95, 96]. Associated with VP5 scaffolds are additional key components including hundreds of copies of heterotrimeric (triplex) VP19-VP23 cement lashed between the VP5 hexamers and pentamers, the 12 kDa hexon-capping protein VP26, and heterodimeric pUL17/pUL25, which binds to triplexes that are adjacent to pentons [97]. Beyond this, in the space between the capsid and the envelope, is the tegument of at least 23 viral encoded proteins that serve a number of functions during entry, replication, and egress [98].

The majority of tegument proteins progressively dissociate from the capsid when the viral envelope fuses with either the plasma membrane or an endosomal membrane. Known exceptions of nondissociating tegument proteins are pUL36 and pUL37 which remain associated with the capsid during transport to the nucleus [99, 100]. To reach the nucleus, HSV1 uses HSP90 enhanced dynein-mediated microtubule minus end-directed transport, but precisely how this is orchestrated is not well understood [101]. VP26 has been shown to interact with dynein light chains RP3 and Tctex1 but binding is dispensable for transport to the microtubule-organizing center (MTOC) [102, 103].

From the MTOC, pUL36 targets NPCs via a NLS motif adjacent to its N-terminal hydrolase domain [104]. NPC attachment is importin-b dependent and involves pUL36 and pUL25 interactions with FG repeat sequences in Nup214 and Nup358 [99, 105, 106]. Capsid component pUL25 and tegument pUL36 are located beside the pUL6 portal in the intact capsid, and thus these interactions likely help to properly align capsids at NPCs for genome import. The viral portal upon receiving appropriate signals will open and with concerted involvement of pUL36 and pUL25 the highly pressurized DNA gets released from inside the capsid where it has been concealed in a tightly wound coaxial spool configuration [105, 107, 108]. The high internal pressure of tens of atmospheres is enough to eject a large fraction of the genome from the capsid, and this may be enough for a part of the genome to forcefully overcome the NPC permeability barrier to enter into the nucleus [109, 110].

3.4 Simian Virus 40

Simian virus 40 (SV40) particles are comprised of VP1 chains that interweave and interlock to form a sturdy 50 nm capsid surrounding a ~ 5 kbp double-stranded circular DNA genome, which gets packaged into a chromatin-like structure with cellular histones H2A, H2B, H3, and H4 [111, 112]. The minor capsid proteins VP2 and VP3 serve as alternating anchor points for tethering the viral genome to the VP1 shell [113, 114].

Endoplasmic reticulum (ER) localization is an essential feature of the SV40 life cycle as incoming capsids must make use of ER-resident factors in order to uncoat [115]. ER luminal proteins, including disulfide reductases ERdj5 and ERp57, along with protein-disulfide isomerase, work cooperatively to isomerize specific interchain disulfides (C9-C9 bond is reduced and reforms as C9-C104) in neighboring VP1 molecules, resulting in the release of a

subpopulation of pentamers from the SV40 capsid [116, 117]. Additionally, these host-induced conformational changes expose the minor capsid proteins VP2 and VP3, generating a hydrophobic SV40 particle that is capable of associating with the ER membrane [118].

For ER-to-cytoplasm transport, SV40 recruits HSP70 BiP, which holds the virus in a transport-competent state until the appropriate time when nucleotide exchange factor Grp170 induces nucleotide exchange of BiP, releasing virus for immediate transport through the ER membrane [119, 120]. The penetration step occurs at specific foci where membrane-bound proteins (B12, B14, C18, and BAP31) facilitate ER-associated degradation (ERAD) retrotranslocation to the cytoplasm [121].

ER released capsids undergo further disassembly as calcium ions dissociate from VP1 pentamer interfaces and interchain disulfides are destabilized in the reducing and low-calcium environment of the cytosol [116, 122]. It is at this time that interiorly situated NLSs on genome associated VP2 and VP3 become exposed and are recognized by importin-a/b heterodimers for transport through NPCs [113, 123, 124]. Although active transport involves VP2 and VP3, it appears likely that a final uncoating event happens while the viral nucleoprotein complex shuttles along the NPC central channel axis based on the observation that viral DNA emerges in the nucleoplasm devoid of any capsid components [125].

3.5 Adeno-associated Virus Type 2

The ~ 4.7 kb single-stranded genomic DNA of adeno-associated virus type 2 (AAV2) is made up of two genes that express four replication proteins and three capsid components via a helper-dependent viral life cycle that requires the presence of a helper virus such as adenovirus or herpesvirus [126]. The three structural subunits are synthesized in a 1:1:10 ratio (VP1, VP2, and VP3, respectively) from a single open reading frame but they differ in their N-terminal extensions due to differential splicing (VP1) and alternative translational start sites (VP2 and VP3). Each of the coat proteins share a common structural motif, the jelly-roll β-barrel comprised of two antiparallel β-sheets, and 60 modules assemble together into a 26 nm T = 1 icosahedral shell characterized by having three elongated spikes surrounding the three-fold axes and narrow empty channels at the five-fold axes [127].

AAV2 transport from cell surface to nucleus involves the so-called clathrin independent carrier/GPI-anchored-protein-enriched early endosomal compartment (CLIC/GEEC) pathway for tSNARE syntaxin 5 meditated retrograde trafficking to the trans-Golgi network (TGN)/Golgi apparatus [128, 129]. Escape to the cytoplasm is dependent on the endosomal-to-TGN transport machinery and exposure of active phospholipase A2 (PLA2) domain in the VP1 N-terminus [130]. Timely extrusion of PLA2 through the channels at the capsid five-fold vertices appears to be triggered by pH- and proteolytic cleavage-induced conformational changes, whereas activation of PLA2 occurs only after AAV2 particles have entered the calcium-rich compartments of the TGN/Golgi apparatus [129, 131-134].

Capsids released into the cytoplasm remain intact, but have undergone discrete conformational changes that expose three N-terminally located basic amino acid clusters with nuclear localization activity in capsid proteins VP1 and VP2 [135, 136]. These clusters

interact directly with impβ, which along with impα proteins and importin-7 facilitate NPC translocation. But how cytoplasmic capsids reach the NPC has remained unknown.

Because the AAV2 capsid is only 26 nm in diameter, it can in principle enter the nucleus intact and then uncoat and release the viral genome. Superresolution data reveal that approximately 17% of incoming AAV2 capsids appear to traverse NPCs with an average import time of 54 ± 29 ms (half that measured for abortive events) suggesting that NPCs or upstream transport or uncoating steps may be a bottleneck to AAV replication [137].

3.6 Human Immunodeficiency Virus Type 1

The envelope spike gp120/gp41 of human immunodeficiency virus type 1 (HIV1) uses CD4-induced conformational changes to promote productive cell surface attachment and to facilitate energetically stable fusion between viral and host cell membranes, which results in the release of capsid cores into the cytosol of mainly $CD4^+$ T lymphocytes and macrophages [138, 139]. Mature fullerene cone-shaped cores consist of approximately 1500 copies of capsid protein (CA), which organize as linked hexamers to form a hexagonal surface lattice that is acutely bent into canonical form by incorporation of 12 CA pentamers [140]. Inside the capsid resides the viral genome, a 9.2 kb homodimer of single-stranded RNA, plus viral enzymes, such as reverse transcriptase, integrase, and protease.

The CA coat is semi-permeable creating a protected microcompartment for reverse transcription during cytoplasmic transit [141]. Each CA hexamer contains a central, size-selective, strongly electropositive (arginine-rich) pore that opens with iris-like motion for rapid uptake of nucleotides so that genomes can be reverse transcribed in viral shells while they move along microtubules in a dynein/kinesin-1 dependent, MAP1/FEZ1 promoted manner [141-143]. Nascent polymerized viral DNA likely induces considerable stress on incoming capsids by the time they reach the nucleus.

Binding of HIV1 capsid cores to NPCs involves interaction between the N-terminal surface exposed CypA binding loop in viral CA and the cyclophilin domain at the tip of filament Nup358 [144]. Other FG regions of Nup358 may play a role in docking HIV1 at the cytoplasmic face of NPCs, however, experiments have ruled out involvement of adjacent Nup214 [145]. Uncoating of NPC-anchored virus requires intact microtubules and appears to be driven by concerted actions of Kif5B and Nup358 [146, 147]. Such action, akin to Ad5 capsid disruption by Kif-5C [60] may provide the force necessary to destabilize and downsize the core for passage through the NPC, but may not fully remove all of the CA from virus particles [148, 149].

CA-associating factors CypA and CPSF6 may play a critical role in guiding interactions between HIV1 and nuclear import components including Nup358, transportin-3, and Nup153 [150-152]. Following arrival at the nucleoplasmic side of the NPC, Nup153 assists HIV1 with exiting the nuclear basket and with targeting transcriptionally active chromatin possibly in concert with Nup98 [145]. Recent EdU click-labeling immunofluorescence experiments have shown that over half of HIV1 cDNA accumulates as capsid-free dead-end products in the cytoplasm during infection of primary human monocyte-derived macrophages (MDM) [149]. Some fraction of these capsid-free genomes may be misdelivered at the stage of NPC

transport. One intriguing possibility that is currently under debate is that interferon-induced myxovirus resistance protein 2 (also referred to as MxB) restricts HIV1 at the NPC by targeting the capsid lattice in a manner that inhibits uncoating at the NPC or import through the FG-lined central channel [153-157].

3.7 Autographa Californica Multiple Nucleopolyhedrovirus

Autographa Californica Multiple Nucleopolyhedrovirus (AcMNPV) infects a wide variety of invertebrates (e.g., moths, mosquitoes, and shrimp) with a double-stranded circular DNA genome of approximately 130 kbp in size that contains more than 150 open-reading frames. The viral replication cycle is complex, and involves two types of virions: one is occluded and adapted for stability outside of the host and initiates infection in midgut cells (columnar epithelial and regenerative cells), whereas the other is a budded virion that is critical for cell-to-cell spread [158].

The nucleocapsid of AcMNPV is rod-shaped and consists of an apical cap, a cylindrical sheath, and a basal structure [159]. Sheath assembly is catalyzed on the basal structure and apical cap mediates genome insertion into preformed capsids [160]. The tubular capsid shell is composed of nine or more proteins with the major structural building block being VP39. Viral DNA undergoes protamine-induced condensation by associating with dephosphorylated 6.9K protein to fit inside preassembled capsids, and later release at the site of replication in the nuclear compartment occurs following rephosphorylation of 6.9K by a capsid-associated kinase [161-163].

Viruses considered thus far move intracellular by microtubule-based mechanisms or diffusion, but AcMNPV uses actin-based motility [164]. Here, cytoplasmic nucleocapsids cause actin to polymerize at one end of the virion, the basal structure, when VP78/83 directly activate the actin nucleation Arp2/3 complex [165]. Activation results in rapid emergence of fishbone-like arrays of actin filaments with plus ends oriented toward viral capsids so that fast polymerization causes forward movement. This is akin to actin tail formation on cytoplasmic listeria, shigella, or rickettsia bacteria, as well as on the plasma membrane underlying extracellular vaccinia virions [166-168].

Exactly how AcMNPV targets NPCs for transport is not understood but translocation of intact nucleocapsids (30 x 250-300 nm) appears to occur independently of the Ran-GTPase cycle, and requires actin-based propulsion mediated by Arp2/3, as well as nucleocapsid-associated Ac132 [169, 170]. Nucleocapsids cross NPCs lengthwise with the distal end containing the actin-assembly inducing VP78/83. It is currently thought that actin propulsive forces are strong enough to breach the FG permeability barrier of the central channel [171].

3.8 Hepatitis B Virus

Hepatitis B virus (HBV) possesses one of the smallest known fully replication competent genomes among animal viruses – a partially double-stranded DNA molecule of less than 3,200 nucleotides in length. The infectious enveloped virion is a spherical particle of approximately 42 nm in diameter, with an inner genome-containing icosahedral capsid composed of a single polypeptide called the core antigen (HBcAg). HBcAg polypeptides are

rich in α-helices and dimerize by pairing of α-helical hairpins between neighboring subunits to form a four-helix bundle fold that is in a capsid [172-174]. In this way, dimeric HBcAg spontaneously self-assembles to form the protective shell surrounding the viral genome.

Initially, a HBV genome is added to assembling shells as single-stranded RNA entities that get retro-transcribed within the core, akin to incoming HIV capsids [141], leading to synthesis of circular dsDNA from the linear pregenomic RNA [175-177]. The local packing of subunits in HBV virions is such that there are large pores in the capsid surface that allow nucleotides to diffuse in and out [178, 179]. Afterwards DNA-containing capsids become enveloped by membranes containing viral glycoprotein surface antigen, of which there are three size variants the large (L), medium (M), and small (S) proteins, to yield the completely assembled and infectious virion [180-182].

Infection occurs by endocytic uptake and pH-independent membrane fusion, after which, capsids are released to the cytoplasm where they use active microtubule-mediated transport to accumulate at the NE [183, 184].

HBV nucleocapsids, like AAV2 and AcMNPV, are small enough in diameter (27 nm) to pass directly through the NPC and do so by interacting with nuclear transport receptors importin-a and importin-b [185]. Here, phosphorylation of HBcAg capsid protein and/or genome maturation appears to induce exposure of two C-terminal arginine-rich NLSs that mediate binding and translocation of intact virus particles at NPCs via the importin-a/b pathway [185, 186]. Translocated HBV-TR complexes bind to nuclear basket Nup153, after which disassembly proceeds by an unknown mechanism, resulting in the release of viral genomes and their accompanying polymerases into the nucleus [186]. Notably, uncoating is restricted to capsids with a mature genome, whereas so-called immature virus is able to cross the NPC but remains trapped in the nuclear basket until genomes are ‘matured’ for release. This phenotype is reminiscent of immature adenovirus TS1 particles which fail to initiate their uncoating process and do not activate their membrane lytic protein VI owing to lack of sufficient internal pressure to react to the mechanical uncoating cues from the surface receptors of the virion [7, 8].

4. Conclusions

Understanding how viruses dock and deliver at NPCs will continue to be an exciting and challenging topic to study for years to come. Based on a rich record of virus research in cell biology, it will yield new and unexpected information about NPC structure and function, as well as inform new ways of controlling viral infections to improve human and animal health. Over the past decades, progress in understanding viral NPC transport mechanisms has been spectacular, however the story is still incomplete and key questions remain. For example, does the NPC scaffold undergo major structural rearrangements to widen for passage of large viral cargoes and if so, what are the molecular signals that trigger conformational change? Also, from a virology and gene therapy point of view, what are the causes and consequences of viral genome misdelivery at NPCs for both viruses and host cells? To advance our knowledge of virus-NPC interactions and their dynamics, scientists from different

backgrounds working collaboratively will span resolutions ranging from the atomic level up to that of the cell.

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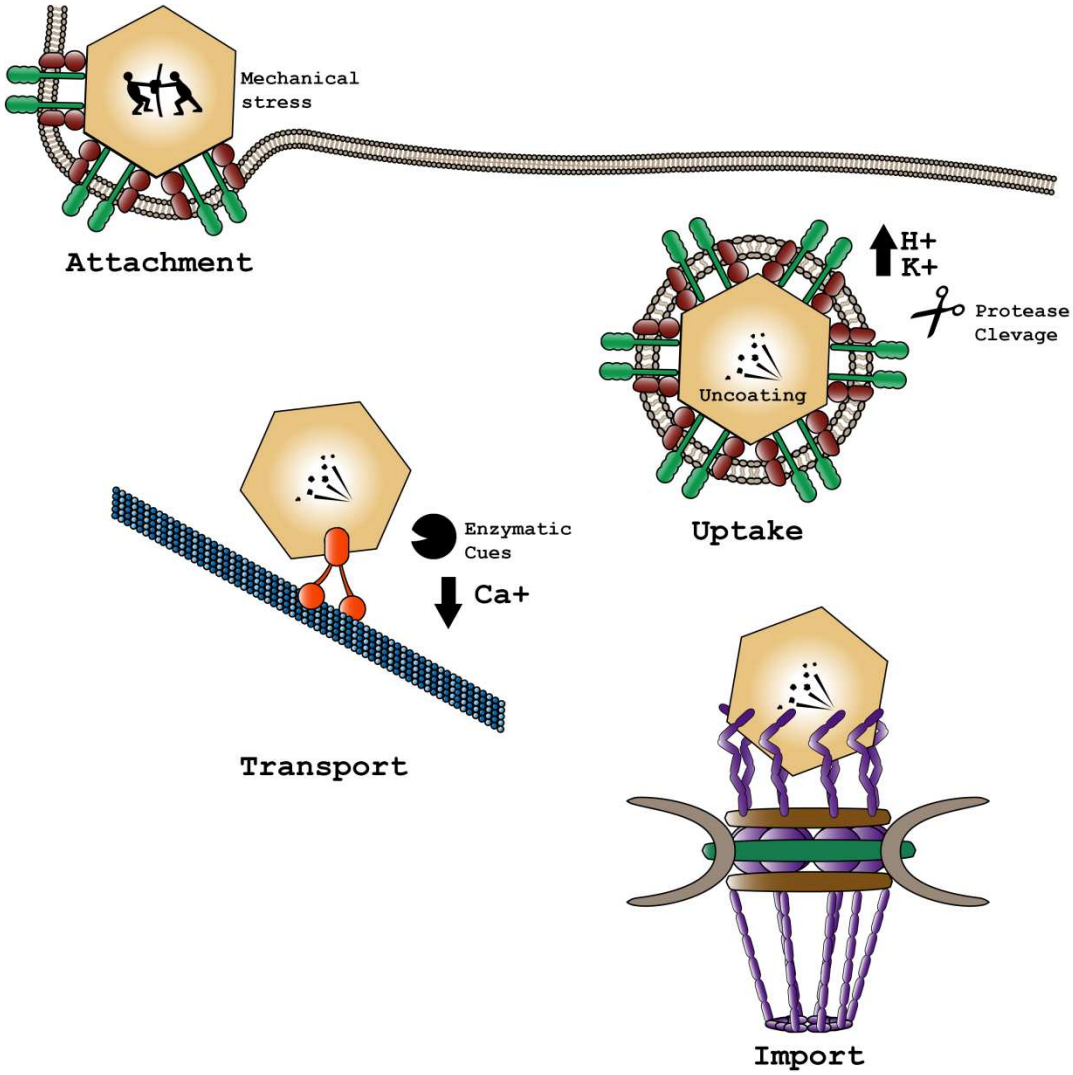

Fig. 1. Preparing for delivery at the NPC. During cell entry, capsids will uncoat in a stepwise gain-of-function process termed uncoating that primes incoming virus particles for docking at the NPC. Initial interactions with surface receptors introduce mechanical stress and initiate capsid disassembly, which continues under the guidance of cellular cues during endocytic uptake, cytoplasmic trafficking, and in some cases, even after attaching to the cytoplasmic face of the NPC.

Table 1: Viral and cellular requirements for reaching the NPC

Virus Particle	Cellular attachment	Uptake Route	Intracellular Transport	Genome Uncoating
Adenovirus type 5 (Ad5)	Ad5 fiber knob binds CAR [187]. Ad5 penton base RGD binds α_v integrins [188].	Particles enter mainly via clathrin-mediated endocytosis and disrupt endosomes via protein VI [189, 190].	Hexon HVR1 binds dynein for movement towards the NPC [53].	Detachment of fiber and weakening of the capsid vertex regions requires CAR, integrins, and actomyosin-2 drifts [77]. Final disassembly occurs at the NPC and is mediated by protein IX, Nup358, and the light chain of kinesin-1 [59, 60].
Influenza A Virus (IAV)	Hemagglutinin (HA) binds to sialic acids (e.g., α2,6-linked) [191].	Particles gain access mainly through clathrin-mediated endocytosis [192].	Intracellular transport involves diffusion/short-range movements along actin [94, 193, 194].	Endosomal destabilization starts when H^+ and K^+ flow through M2 channels. After fusion, the eight viral ribonucleoproteins (vRNPs) disassociate from the matrix using unanchored ubiquitin chains, which activate the aggresome pathway, and with the help of cytoskeletal motors, completes uncoating [74, 195].
Herpes simplex virus type 1 (HSV1)	gB/gC use heparin sulfate for attachment [196]. gD binds HVEM, nectins, or sites in heparin sulfate generated by 3-O-sulfotransferases [197].	Virion envelopes fuse with host membranes in a process driven by a large structural rearrangement of the trimeric fusogen gB, with help from gD and gH/gL [198-200].	Dynein and dynactin are required for efficient capsid transport along microtubules to the nucleus [201].	Post-fusion content mixing initiates shedding of a large portion of tegument away from the DNA-containing capsid, although at a few tegument proteins remain associated all the way to the nucleus. Upon arriving at the nucleus, a single vertex opens and releases the dsDNA with help from pUL36 and pUL25 [107, 108, 202].
Simian virus 40 (SV40)	SV40 uses the GM1 ganglioside, a branched glycan carrying α2,3-linked sialic acid on one of its two branches as its cellular receptor [203].	Uptake occurs via endocytic organelles until transfer into the ER lumen [204].	Upon penetrating the ER and reaching the cytoplasm, viral DNA is escorted to the nucleus by the NLS-containing capsid proteins VP2 and VP3 [123, 124].	The SV40 capsid doesn't seem to change until entering the ER lumen where proteins such as ERp57, ERdj5, and protein-disulfide isomerase induce structural rearrangements that expose a hydrophobic stretch critical for BAP3-mediated ER penetration. Escape to the cytoplasm is followed by loss of interchain disulfides and calcium depletion, which causes the VP1 capsid to fall apart [115-122].
Adeno-associated virus type 2 (AAV2)	AAV2 targets HSPG along with co-receptors: AAVR, FGFR, HGFR, 36/67 kDa lamin receptor, or $\alpha_v\beta_1$/$\alpha_v\beta_5$ integrins [205-210].	Virus is taken up via CLIC/GEEC. Transport to cytoplasm depends on the endosome-to-TGN transport machinery and exposure of PLA2 in theVP1 N termini [128, 211].	Modes of transport include dynein-mediated and microtubule-independent processes [212, 213].	Structural changes have not been observed during early entry. In endosomes, VP1 undergoes small conformational changes brought on by cleavages (cathepsins B and L) and by acidification, which for one, helps expose the PLA2 sequence, needed for TGN/Golgi apparatus escape. Cytoplasmic AAV2 is small enough (~ 26 nm diameter) to pass through the NPC central channel intact. Uncoating happens after nuclear import [214, 215].
Human immuno-deficiency virus type 1 (HIV1)	HIV1 envelope glycoprotein (ENV) binds to CD4 and the chemokine co-receptor CCR5 or CXCR4 on the cell surface [216].	gp120 binds CD4 and rearranges to engage co-receptor, allowing the distal tip of gp41 to insert and mediate fusion at the edges of cholesterol-rich lipid domains in the host membrane [139, 217].	Moves using kinesin-1 and dynein. The process is regulated by FEZ1 and promoted by MAP1 [142, 143, 146].	The HIV capsid remains largely intact as it traverses the cytoplasm, and it is during this time that nucleotides are imported through dynamic, selective pores in the CA hexamers for reverse transcription. Nascent, polymerized viral DNA likely induces stress on the structure of the capsid, which when bound at the NPC, appears to uncoat by a Nup358/kinesin-mediated 'tug of war' like mechanism [141, 218].
Autographa californica multiple nucleopoly-hedrovirus (AcMNPV)	The receptor has not yet been described, but it is thought that gp64 binds to heparin sulfate or a surface phospholipid [219, 220].	Virus is primarily internalized via clathrin-mediated endocytosis. Drop in pH triggers gp64, which is a class III fusion protein, acting similar to HSV1 gB [221].	The nucleocapsid is propelled through the cytoplasm by generating a fishbone-like array of actin filaments [165].	The capsid does not uncoat before nuclear import, but rather docks at the NPC and passes through the central channel intact, after which uncoating of the circular, supercoiled DNA occurs [171].
Hepatitis B virus (HBV)	HBV first binds HPSG and then the pre-S1 domain of envelope protein L interacts with the liver bile transporter, NTCP [222-224].	Caveolin-1 and clathrin-mediated pathways lead to infection. Fusion occurs at plasma or endosome membranes and cholesterol is important for escape [225-227].	Following fusion of viral envelope with membrane, nucelocapsids are actively transported via microtubules to the nucleus [228].	The HBV capsid is small enough to pass through the NPC intact, which afterwards, the genome is released in a regulated fashion [185].

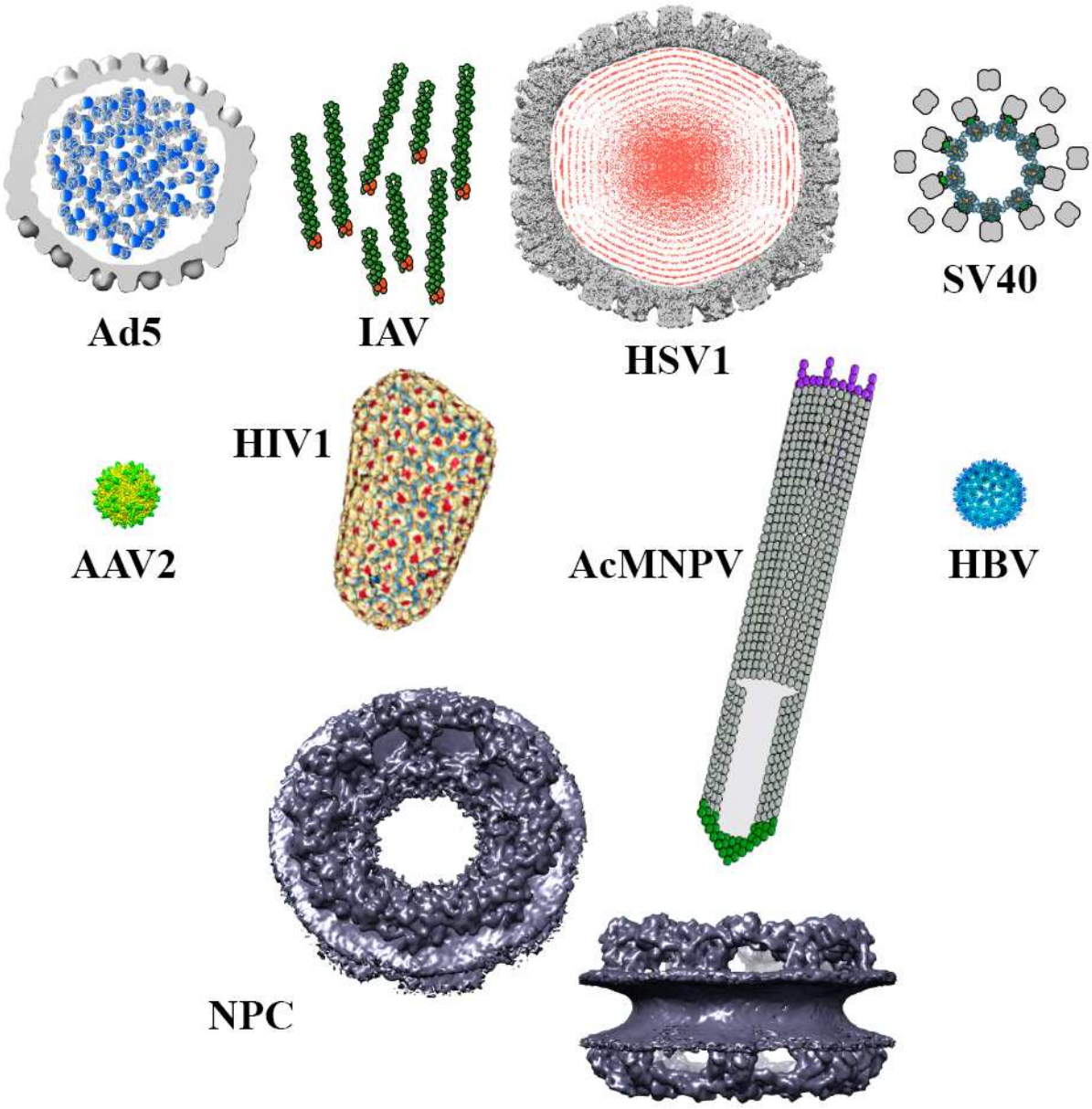

Fig 2. Viruses of different shapes and sizes transport genomes through NPCs. The eight viruses discussed in this review are shown to scale with the NPC. Ad5, HSV1 and HIV1 use so-called forced break-in to translocate nucleic acids into the nucleus, whereas IAV, SV40, AAV2, AcMNPV and HBV are shape fitting. Ad5 is depicted as a cross-section of cryo-EM density with a stylized core of dsDNA packaged with viral proteins adapted from [47]. IAV is shown as negative-sense RNA devoid of M1. HSV1 (EMD- 6386) was visualized in chimera with protein-free dsDNA colored red. SV40 is displayed as a disulfide reshuffled weakened capsid enclosing circular dsDNA packaged with cellular histones. AAV2 capsid is based on the work of Kronenberg et al. [229]. HIV1 nucleocapsid (PDB: 3J3Q) is represented using NGL viewer [230]. AcMNPV is a bullet-shaped with a polarized actin nucleation site (purple). HBV capsid is based on the work of Wang et al. [231]. The NPC structure is based on the work of Eibauer et al. [20]. The central channel of the NPC is empty in the displayed cryo-ET reconstruction as it contains the permeability barrier made of natively unfolded FG proteins.